*Kwik*Western Buffer

(Cat.# R2002)

Instrument

Shaker: An orbital or linear platform shaker. A gentle rocker is not preferred.

Setting: 50-80 rpm. It's essential that the membrane moves freely inside the tray in every step. Otherwise, the blot may have a high background.

Procedures

(All steps use at least 0.5 mL/cm^2 membrane. Do not use less buffer volume. To minimize plastic waste, use a graduated tube to measure the buffer volume, and re-use it.)

1. Make 4% skim milk solution using R2002. Immerse the membrane in it for 30 min. After that, rinse the membrane two times with R2002.
2. Dilute your primary antibody in R2002 (see note*), and add to the membrane.
3. Incubate on the shaker for 1-24 hours at room temperature. The antibody is stable in this buffer.
4. Recover the primary Ab solution and store it at 4^{o}C for future reuse. The Ab solution can also be stored at -20^{o}C for years. Typically the antibody solution can be re-used at least 5 times. Once the signal starts to go down, spike the solution with the same antibody to make up for the loss of antibody.
5. Rinse the blot using R2002 two times, each for 15 seconds. Then wash the membrane on the shaker for 1 hour at room temperature. Discard the wash buffer and add the secondary antibody in the next step.
6. Dilute the Digital HRP-secondary antibody (R1005-1009, R1011-1012) in R2002, at 1:1000 ratio. Then add to the blot in step 5. Incubate the membrane on the shaker for 1 hour at room temperature. (If the user uses his/her own secondary Ab, the user may need to adjust the antibody dilution factor for optimal results.)
7. Rinse the blot using R2002 two times, each for 15 seconds. Then wash the membrane on the shaker for 1 hour at room temperature.
8. Rinse the blot with de-ionized water for 15 seconds to remove residual R2002, and transfer the blot onto the sample tray of ***Kwik*Quant** Pro Imager (D1010).
9. Apply 1:5 H_2O-diluted ***Kwik*Quant** Digital-ECL (R1002) mixture (A:B=1:1) on the membrane and wait for 2 minutes.

10. Drain the surface ECL solution, and immediately put the membrane in ***Kwik*Quant** Pro Imager for image acquisition.
11. If the bands are too weak at 2 min exposure, re-apply the non-diluted ***Kwik*Quant** Digital-ECL mixture (A:B=1:1) on the membrane for another 2 minutes, followed by image acquisition.
12. If the blot shows a background, put the membrane in R2002 and wash for another 2 hours at room temperature.
13. After the image acquisition, the membrane can be stored temporarily in deionized water at 4°C. The image acquisition can be repeated within 24 hours without significant loss of signals. The membrane can also be frozen at -20°C in water for a longer period.

Note*: The primary Ab may need 50%-90% further dilution than that normally diluted in skim milk. For example, if the primary Ab is normally used at 10µg/ml in 4% skim milk, users may further dilute it to 2µg/ml in R2002. This is because skim milk often suppresses antibody-antigen interaction. The optimal dilution factor may be determined empirically.

Buffer Preparation

Method 1 (for occasional users): Dissolve components A and B in 5L de-ionized water in two separate containers and store them at room temperature. The solutions are stable up to 12 months. Mix A and B solutions at 1:1 (V/V) before use.

Method 2 (for frequent users): Dissolve components A and B in 10L de-ionized water in one container (Cat#. D2002). The buffer is good for 1 month if stored at room temperature.